

PHARMACEUTICALLY ACCEPTABLE INTRAMOLECULARLY CROSS-LINKED, STROMAL-FREE HEMOGLOBIN

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 553,514 filed on Feb. 2, 1975, now U.S. Pat. No. 4,001,401 and U.S. patent application Ser. No. 554,051, filed on Feb. 2, 1975, now U.S. Pat. No. 4,001,200. This application and the related applications are assigned to the same assignee, and benefit of their filing dates is claimed herein.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to novel and useful (1) intramolecularly cross-linked, stromal-free deoxyhemoglobin, and (2) intramolecularly cross-linked, stromal-free oxyhemoglobin. The intramolecularly cross-linked stromal free hemoglobins of this invention have the property of reversibly binding gaseous ligands such as oxygen, and they are useful as blood substitutes for transporting and supplying oxygen to vital tissues and organs, as blood plasma substitutes as sources of oxygen for biological systems, and for furnishing oxygen to oxygen-consuming environments.

2. Description of the Prior Art

Hemoglobin is present in the blood of mammals and it has the fundamental property in solution of reversible oxygenation. In its natural form, mammalian hemoglobin is a conjugated, non-crosslinked protein having a molecular weight of 64,500 and structurally comprised of two pairs of sub-units. Each sub-unit contains a heme group and a polypeptide chain, called globin. In mammals, hemoglobin is present in erythrocytes along with stroma which consists of proteins, phospholipids and cholesterol. *Clinical Hematology*, by Wintrobe, 6th Ed., pages 138 to 199, 1967, published by Lea and Febiger, Philadelphia, Pa. The reaction of isolated, bovine hemoglobin containing stroma with glutaraldehyde is known to the art in *Histochemical J.*, Vol. 2, pages 137 to 150, 1970, wherein Hopwood used an excess of glutaraldehyde to obtain an insoluble precipitate. Similarly, the reaction of whole blood proteins with glutaraldehyde leading to a water insoluble glue is disclosed by Karjala, et al, in the U.S. Pat. No. 3,294,564. The interaction of the collagen and collagen degradation product gelatin with diisocyanates and other polycoupling agents, including aldehydes, is reported by Campbell in U.S. Pat. No. 2,591,133, by Linder, et al, in U.S. Pat. No. 3,057,782, and by Bowes in *Biochimica et Biophysica Acta*, Vol. 168, pages 341 to 352, 1968. The carboxyalkylation of globin for use as a plasma expander is taught by Biddison in U.S. Pat. No. 2,719,837; however, the products obtained by the above reactions lacked the ability to transport oxygen and in consequence thereof, they did not enter into general use. In U.S. Pat. No. 2,527,210, Bower disclosed the use of hemoglobin for treating wounds, in U.S. Pat. Nos. 3,000,836 and 3,519,572, Ginsburg and Kita disclosed blood preparations having utility as standards for measuring hemoglobin. In *Federation Proceedings*, Vol. 34, pages 1458 to 1460, 1975, Mok et al. disclosed the reaction of hemoglobin solutions containing stromal-free protein with diimidate esters leading to intramolecularly cross-linked and intermolecular complexes. In U.S. Pat. No.

3,925,344, Mazur disclosed a plasma protein substitute consisting of intramolecularly cross-linked hemoglobin formed by using diimidate cross-linking agents. In these latter two references, the reaction was carried out apparently in an air environment and free of reducing conditions which leads to hemoglobin in the oxygenated form. A diimidated cross-linked hemoglobin similar to the product of U.S. Pat. No. 3,925,344 also is disclosed in Netherland Pat. No. 7,041,140.

OBJECTS OF THE INVENTION

It is an immediate object of this invention to provide both intramolecularly cross-linked, stromal-free deoxyhemoglobin and oxyhemoglobin useful as blood substitutes and blood plasma expanders possessing enhanced physical and chemical integrity in the environment of use.

Still a further object of the invention is to provide both intramolecularly cross-linked, stromal-free deoxyhemoglobin and oxyhemoglobin useful as blood substitutes and blood plasma expanders and having increased intravascular persistence over a prolonged period of time.

Yet a further object of the invention is to provide new and useful therapeutic agents useful as blood substitutes and blood plasma expanders that are miscible with blood and its components, and are substantially non-toxic, non-antigenic and non-pyrogenic.

Yet still a further object of the invention is to provide novel blood substitutes and blood plasma expanders that are readily available, stable under prolonged storage and are safely discharged from circulation.

Still another object of the invention is to provide blood substitutes that can transport and supply oxygen to vital tissues and organs.

Yet still a further object of the invention is to make available both intramolecularly cross-linked, stromal-free deoxyhemoglobin and oxyhemoglobin solutions having an osmolality substantially equal to whole blood with utility as a volume replenisher.

Yet still another object of the invention is to make available to the art both intramolecularly cross-linked, stromal-free deoxyhemoglobin and intramolecularly cross-linked, stromal-free oxyhemoglobin possessing colloid and oncotic properties useful for maintaining the level of blood and plasma in the management of disease.

Still a further object of the invention is to make available new compositions of matter, comprising both intramolecularly cross-linked stromal-free deoxyhemoglobin and oxyhemoglobin that can take over certain functions of plasma and have the oxygen carrying function of red blood cells.

Still a further purpose of the invention is to provide new biologically active molecules of defined and homogenous molecular weight that are soluble in physiological fluids and can be used as a blood substitute and blood plasma expander without an accompanying risk of transmission of disease.

These objects as well as other objects, features and advantages of this invention, will become more readily apparent to those skilled in the art from the following detailed description, the drawings and the accompanying claims.

SUMMARY OF THE INVENTION

This invention concerns both an intramolecularly cross-linked, stromal-free deoxyhemoglobin, and an intramolecularly cross-linked, stromal-free oxyhemo-